OxfordMaterials

In the Matter of US Patent Application
Serial Number 09/720,411 CZERNUSZKA et al.

DECLARATION

I, JAN TADEUSZ CZERNUSZKA, declare as follows:-

1. I am a British citizen employed by the University of Oxford, Department of Materials, Parks Road, Oxford, OX1 3PH and I am the first named inventor of the above referenced application.

2. Since 1996 I have been a University lecturer at the Department of Materials, University of Oxford and a Fellow and Tutor in Materials at Trinity College, Oxford. I possess a BSc. (Hons), ARSM in Materials Science from Imperial College, London, an MA from the University of Oxford and a Ph.D from the Department of Metallurgy and Materials Science, University of Cambridge. I have published over 100 articles on biomaterials, material and microscopy and related areas.

3. I have been asked to review the procedure used by E. D. Eanes to prepare vesicles as discussed in Calcified Tissue International (1987) 40: 43-48 and Bone and Mineral 17 (1992) 269-272 .

4. Eanes prepares vesicles using a phospholipid, typically phosphatidycholine. Put briefly, a mixture of the lipid was buffered with phosphate and then rotary-evaporated. Liposomes (also known as vesicles) were obtained while hydrating the liquid film with gentle shaking and then with more vigorous shaking. The liposomes which were formed were then suspended in a buffered solution containing calcium and phosphate ions. In some experiments an ionophore, lasalocid acid, sodium salt, was added.

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5. It is clear that the liposomes or vesicles obtained contained phosphate. Indeed it is clear from the reference to ionophore-mediated Ca^{2+} fluxes from the external suspending medium in the Calcif. Tissue article, page 43, right hand column that the purpose of adding the ionophore is to make the liposomes permeable to calcium so that calcium can penetrate the walls of the vesicles and act as a seed for the formation of calcium phosphate on the external surface of the phospholipid walls. There is no suggestion anywhere that I can see that Eanes was able to deposit a layer of calcium phosphate on the outside of the lipid layer without seeding through the vesicle walls.

6. In my experience I can say that the resulting vesicles would have no practical value as carriers for pharmaceutically active compounds which can be released over time, typically as the walls of the vesicles are dissolved by the surrounding medium. This is because the walls of Eanes' vesicles are ruptured by the calcium phosphate. This means that any pharmaceutical present inside the vesicles will leak out in an uncontrolled manner over a short space of time. If vesicles are to be of any real value for the administration of a pharmaceutical agent they must, of course, release the pharmaceutical agent in a predetermined and controlled manner. The vesicles of Eanes cannot do this by virtue of the fact that the membranes are not intact.

I further declare that all statements made herein of my own knowledge are true and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardise the validity of the application or any patent issuing thereon.

29th September 2003
Date

T. C. Eanes
Signature